OBSERVATIONS ON THE MORPHOLOGY AND LIFE-HISTORY OF THE DIGENETIC TREMATODE, *LEPOCREADIUM SETIFEROIDES* (MILLER AND NORTHUP, 1926) MARTIN, 1938 [1]

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Miller and Northup (1926) reported on a one year survey of trematode. infections in *Nassa obsoleta* (Say), taken at Quisset Harbor, near Woods Hole, Massachusetts. They examined 8,875 snails and described five new species of cercariae. The incidence and intensity of infection of each species were recorded for each month. One of these species, an ophthalmotrichocercous distome larva, was designated *Cercaria setiferoides*. Both redial and cercarial generations were described.

Martin (1938) redescribed the species and conducted experiments to disclose the life-cycle. The cercariae were recognized as members of the genus *Lepocreadium* Stossich, 1904 and the species was named *Lepocreadium setiferoides* (Miller and Northup, 1926).

Peters (1961) studied the development of the excretory system in the cercariae of species in the Lepocreadiidae and Acanthocolpidae. As the representative of lepocreadid cercariae, he selected *L. setiferoides*.

Although there is sufficient general agreement to assure that these three investigations dealt with the same species, there are serious discrepancies in the accounts. The disagreements are concerned with degree of development of the digestive and reproductive systems, the number of paired setigerous tufts on the tail, the number and arrangement of penetration glands and details of the excretory system. During the summers of 1969, 1970 and 1971, study of *L. setiferoides* has been made in an attempt to resolve the differences in previous accounts and provide additional information on the species. This report presents a redescription of the successive stages in the life cycle and the results of experimental infections in disclosing new secondary intermediate hosts. Finally, a reconsideration of the synonymy restores the validity of *Lepocreadium setiferoides* (Miller and Northup, 1926) Martin, 1938.

OBSERVATIONS AND RESULTS

Life cycle and larval stages

The present study of *Cercaria setiferoides* has provided information to clarify and supplement previous descriptions. Both Miller and Northup (1926) and Martin (1938) described rediae of *C. setiferoides*, and their observations have been confirmed. However, neither reported more than one generation of rediae. There are

at least two generations of rediae. Each daughter redia produces one or more daughters before it begins to produce cercariae.

In living cercariae, twitching of the tail makes counting of the lateral setigerous tufts a frustrating experience, but examination of fixed specimens, killed in hot water, has permitted a certain count; there are 33 pairs and a terminal tuft. The structure of the lateral pairs is similar to that of the "finlets" on the tails of cercariae of *Neopechona pyriforme* as described by Stunkard (1969a). The wall of the tail consists of external circular and inner longitudinal muscles and the central core contains a linear series of large "caudal bodies" or "glycogen cells." The cercariae swim tail first and the action of the paired oar or paddle-like tufts induces rapid progression. The ocelli orient the cercariae with reference to light and swimming movements take them toward the dark side of the container.

The digestive system is well developed. The cercariae emerge from the rediae while immature and complete their development in the haemocoele of the snail. They ingest the snail's blood and their ceca are filled with material that stains intensely with neutral red. When the body is retracted, the material in the ceca is disposed in a spiral coil. The intestinal ceca become narrow posteriorly and communicate with the posterior end of the excretory vesicle, forming a uroproct.

There are eleven pairs of penetration glands; on each side there are three gland cells lateral and posterior to the ocellus; their ducts pass forward lateral to the ocellus. Eight other cells are situated in front of and at the sides of the acetabulum; their ducts pass forward medial to the ocellus. When the anterior end of the cercaria is retracted, certain of the glands nearest the median plane may lie posterior to the digestive ceca, as shown in the figure of Miller and Northup (1926). As a result of pressure from the overlying ceca, the initial portions of the ducts from these cells are empty and not easily observed. There are no cystogenous gland cells and the metacercariae do not encyst.

The reproductive systems are well developed. The testes and ovary are clearly recognizable as described and figured by Martin (1938). When a specimen is retracted, as shown in Martin's figure 3, the testes are opposite but, on extension, pressure of the excretory vesicle causes the testes to be situated obliquely, with the left one slightly anterior to the right. Miller and Northup (1926) described the excretory vesicle and the primary and secondary collecting tubules, but the flame-cell formula was not resolved. Martin (1938) described the sphincter at the posterior end of the bladder, and confirmed the observations on the collecting tubules, but his observations on the pattern and number of flame-cells were admittedly incomplete. Peters (1961) was concerned principally in testing the validity of the taxonomic system proposed by La Rue (1957), which was based primarily on the structure of the excretory bladder and location of the primary excretory pores. The wall of the excretory vesicle is epithelial, composed of cells derived from the mesoderm. In the mature cercaria the primary collecting ducts emerge at the sides of the vesicle and pass laterad along the anterior faces of the testes: then anteriad to the level of the anterior margin of the acetabulum. Here they divide into anterior and posterior secondaries. On each side the anterior branch passes forward to the level of the ocellus where it gives off an anterior tertiary tubule and turns posteriad. The anterior tertiary receives a capillary from a flame-cell located lateral to the ocellus, one from a flame-cell situated immediately anterior to the ocellus, one from a flame-cell that is lateral in position,

and the tubule divides to form two capillaries, one leading to a flame cell immediately posterior to the oral sucker and the other lateral to the sucker. The recurrent branch gives off a second tertiary that divides in a similar manner and drains five flame-cells in the post-ocellar region and a third tertiary that drains five flame-cells in the acetabular region. The disposition of the branches and flame-cells in the posterior half of the body is the exact obverse of that in the anterior half. The location and arrangement of the flame-cells are shown in Figure 1, and the flame-cell formula is $2[(5 + 5 + 5) + (5 + 5 + 5)]$.

Descriptions of cercariae often list maximum, minimum and average sizes of living structure. Such measurements are made under coverglass pressure which varies with the amount of water in the preparation. Length and width of any structure increases as the water evaporates and differences in measurements may vary from 10 to 50 per cent. More accurate and precise figures are obtained by killing the cercariae in hot water before fixation. Standard fixing fluids may follow immediately after killing. This procedure gives specimens of uniform size and shape, which provides a standard for comparison with other species. Cercariae killed in whirling hot water give the following measurements in mm: length of body, 0.225; width of body, 0.115; length of tail, 0.650; width of tail at base, 0.040; acetabulum, 0.040; oral sucker, 0.058; pharynx, 0.021; acellus, 0.012 by 0.015; length of setae, 0.07 shorter distally.

Experimental infections

New experimental work falls into two categories. A variety of invertebrates were exposed to cercarial infection, and subsequently specimens of the species of annelid worms and turbellarians successfully invaded by the larvae were fed to the primary host, the winter flounder, *Pseudopleuronectes americanus.*

The cercariae live for about 48 hours at room temperature; older and somewhat exhausted specimens sink to the bottom where they attach by the tips of their tails. Possible secondary intermediate hosts have been exposed in finger-bowls with large numbers of recently emerged cercariae. Obviously, exposure to hundreds or thousands of cercariae in the confines of a finger-bowl is not comparable to conditions in nature, but failure to obtain infection eliminates any species as a possible intermediate host. The invertebrates employed in exposure experiments often harbored metacercariae of natural infections by other species but the conditions of the experiment made it possible to distinguish between natural and experimental infections, especially since *Cercaria setiferoides* does not encyst. No infections were obtained in crustaceans or in either gastropod or bivalve molluscs, but one surprising observation was to find metacercariae of *Zoogonus lasius* encysted in the limpet, *Acmaea intestinalis.* Of the common scyphomedusae. *Chrysaora quinquecirrha,* was the only species invaded. The ctenophore, *Mnemiopsis leidyi,* which harbors metacercariae of other lepocreadid species, was not attacked. Large polychaete annelids of several families, including *Aphrodita hastata, Arabella iricolor, Clymenella torquata, Glycera americana, Glycera dibranchiata, Lepidonotus squamatus, Nephtys bucera, Notomastus latericeus, Pectinaria gouldii, Scoloplus fragilis,* and *Sthenelais boa* were also immune. But the spionids, *Polydora ciliata* and *Polydora ligni* were susceptible and were attacked vigorously (Figure 2). *Cercaria setiferoides* also invaded the acoelous turbellarian, *Childia* sp. The

identity of the species of *Childia* in the Woods Hole area is uncertain. Hyman (1959) listed *Childia spinosa* Graff, 1917 and *Childia baltica* Luther, 1912 as synonyms of *Childia groenlandia* (Levinsen, 1879) Meixner, 1925. The polyclad turbellarians, *Euplana gracilis* and *Stylochus ellipticus*, were attacked and metacercariae were distributed in the bodies of the turbellarians. The nature of the secondary intermediate hosts probably explains the observations of Linton (1921) and Martin (1938), that infection occurs only in young and small fishes, since older fishes, six to eight inches in length, feed on other and larger organisms.

Laboratory infected intermediate hosts were fed to a small, one-year old winter flounder, *Pseudopleuronectes americanus*, and the fish was autopsied two weeks later. Twenty juvenile specimens of *L. setiferoides* were recovered from the intestine.

Systematic Review and Discussion

It is now worth briefly reviewing earlier work on the life-cycle and systematics of this and possibly allied forms. Miller and Northup (1926) reported about 30 pairs of setigerous tufts on the tail; Martin (1938) found 35 pairs and a terminal unpaired group. Miller and Northup described a well developed digestive tract with wide intestinal ceca extending to the posterior end of the body; they noted the jelly-like contents of the ceca stained intensely with neutral red. Martin (1938) stated, page 465, "The intestine is rudimentary, sometimes appearing as two strings of cells, staining deeply with neutral red, extending from the esophagus to near the posterior end of the body." Miller and Northup (1926) observed, page 499, "Several cell masses in Anlage of reproductive system; no interpretation ventured as to parts of adult system represented." Martin found, page 465, "The reproductive system is well developed. The two testes have slightly lobed or smooth margins and are located in the posterior one-third of the body. The ovary lies slightly to the right of the mid-line between the anterior testis and the ventral sucker." Miller and Northup (1926) found, page 498, "Eleven pairs of larval glands, preacetabular in position, arranged in three groups; no observable ducts from the most posterior six glands; glands of all groups strongly acidophilic in all combinations of stains; lightly stained with intra-vitam neutral red, but not with toluidine blue." Martin (1938) reported "Eight cephalic glands are present on each side of the body and partially surround the ventral sucker. Their ducts pass anteriorly and open to the exterior at the anterior end of the body. These glands stain deeply with neutral red but in the same general region there appear to be other glands that do not absorb this stain. From ten to fifteen cystogenous glands are scattered irregularly through each half of the body." Miller and Northup (1926) described the excretory vesicle with its concretions and the primary and secondary collecting tubules of the excretory system; they reported a succession of single flagella in the wall of the main lateral tubules, and although many flame-cells were observed, the pattern was not resolved. Martin's account of the excretory system is in general agreement; he found 24 flame-cells on each side of the body which appeared arranged in groups of threes. He reported, "However, one of the capillaries of each group joins the secondary collecting tube independently of the other two."

Peters traced development of the excretory system from the condition in the embryo to the definitive pattern in the mature cercaria. The primary excretory
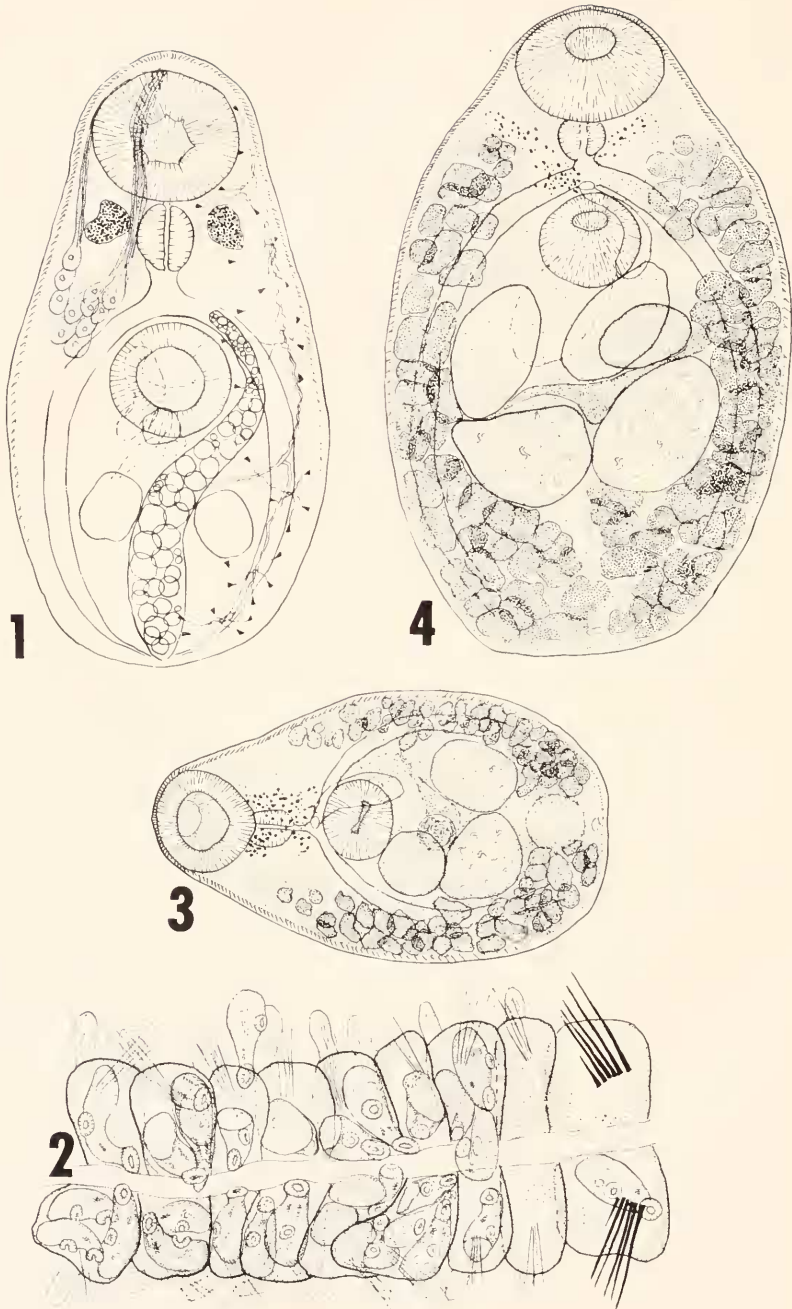
FIGURE 1. *Cercaria setiferoides*, body, drawn from pencil sketches of living specimens, showing penetration glands, Anlagen of the gonads, the digestive and excretory systems, with location of the flame-cells.

pores were lateral, at the body-tail furrow. The primary ducts fused to form the excretory bladder, which elongated as it acquired an epithelial wall. A sphincter was formed at the posterior end of the bladder, followed by a small atrium at the body-tail junction. The flame-cell formula was given as $2[(3 + 4 + 5) + (5 + 5 + 5)]$. Peters observed that as the tail grows, a longitudinal row of conspicuous transitory cells appears in the axis of the tail.

Martin isolated the snails in finger-bowls to check on the emergence of cercariae. He found that they emerge both diurnally and nocturnally; that they are photonegative and swim rapidly by lashing of the tail. The cercariae were observed to penetrate and encyst in the triclad turbellarian, *Procerodes warreni*, and in spionid polychaete annelids. The metacercariae did not increase much in size but the reproductive organs became more mature. After an interval of several days, the infected intermediate hosts were fed to small flounders and sand dabs. *Nereis virens* was used as food for the fishes since it was found that this worm would not serve as a second intermediate host of *L. setiferoides*. Martin noted that the experimental hosts may not be the natural ones, particularly *Procerodes*, which is found in rocky regions that are not the habitats of the flatfish. Natural infections were found in most of the small flounders and sand dabs, so that suitable experimental animals, known to be uninfected with this parasite, were not available. However, the small flounders and sand dabs were fed infected *Procerodes* and spionids over a period of about one month and various stages of development from the metacercariae to the adult were recovered. Martin (1938) found these worms in very small flounders but larger fishes, six to eight inches in length were rarely infected. He stated, page 469, "This probably explains the fact that Linton did not find this species in his extensive study of fish parasites." Martin stated that the adult of *L. setiferoides* had not been described previously, but there is evidence that Linton did observe the juvenile worms and that he described the adults under his account of *Lepocreadium trullaforme*.

Linton (1921) reported on the food of young flounders, *Pseudopleuronectes americanus*, collected from 36 stations in the Woods Hole region from May 2 to November 2, 1915 and 1916. A total of 398 fishes were examined; they varied from 25 to 190 mm in length, although most were less than 100 mm long. The contents of the stomachs and intestines were analyzed. The food of the smallest fishes consisted chiefly of copepods; slightly larger fishes took amphipods and in fishes 40 mm long the intestine contained setae of annelids, probably *Polydora* spp., since these are the only species common in the area and small enough to be ingested by fishes of that size. In the stomachs of small fishes, 30 to 40 mm in length, he found large numbers of appendiculate distomes, hemiurid species that use small crustaceans as intermediate hosts, and slightly larger fishes harbored unidentified distomes, apparently juveniles that very well could have included *L. setiferoides*.

FIGURE 2. *Polydora ligni*, showing the modified fifth setiger and eight succeeding somites, with metacercariae of *L. setiferoides*, exposed one day in finger-bowl.

FIGURE 3. *L. setiferoides*, juvenile specimen from *Menticirrhus saxatilis*, collected 21 August, 1918, by E. Linton; on slide No. 8278 in the Helminthological Collection of the U. S. National Museum; same magnification as Figure 4.

FIGURE 4. *L. setiferoides*, mature specimen, 0.46 mm long, on same slide as Number 3; part of a collection of distomes included by Linton (1940) in his description of *Lepocreadium trullaforme* n. sp.

The new observations, presented above, correct the discrepancies and errors in the previous accounts, reveal the existence of at least two generations of rediae, and disclose several new secondary intermediate hosts, both annelid and turbellarian.

In a posthumous publication, Linton (1940) reviewed, revised and supplemented his earlier studies on the trematodes of fishes mainly from the Woods Hole region, Massachusetts. In this paper he described *Lepocreadium trullaforme* n. sp., to contain worms taken at different times from various species of fishes. These included:

(1) two specimens from the American sole, *Achiurus fasciatus*, reported as *Distomum* sp., in Linton (1901, page 487, Fig. 51); the type specimen is in the Helminthological Collection of the U.S. National Museum under the number 8275.

(2) specimens from the long spined sculpin, *Acanthocottus octodecimspinosus*, taken 22 December, 1906; 12 May, 1913; 20 April, 1914; 26 April, 1915; specimen #8276, U. S. N. M., named as type.

(3) one specimen from the kingfish, *Menticirrhus saxatilis*, taken 11 September, 1907; U. S. N. M., #8277.

(4) many specimens, some immature, from young kingfish, *M. saxatilis*, collected 21 August, 1918; U. S. N. M., #8278.

(5) eight specimens from the white perch, *Morone americana*, collected 8 August, 1910; U. S. N. M., #8279.

(6) specimens from the winter flounder, *Pseudopleuronectes americanus*, described as *Distomum areolatum* Rudolphi in Linton (1900, page 293, Figs. 60–63); U. S. N. M., #6517.

(7) specimens from the cunner, *Tautogolabrus adspersus*, identified as *Distomum areolatum* Rudolphi in Linton (1901, page 462).

As noted, Linton (1940) included *Distomum areolatum* Rudolphi of Linton, 1900 in *Lepocreadium trullaforme* and Yamaguti (1958) suppressed *L. trullaforme* as a synonym of *D. areolatum* Rudolphi of Linton, 1900. Stunkard (1969b) redescribed the species, and showed that it is distinct from *Distomum areolatum* Rudolphi, 1809, and transferred it to *Lepocreadium* Stossich, 1904, as *Lepocreadium areolatum* (Linton, 1900) n. comb. Study of the specimens from the Helminthological Collection listed above, shows that with the exception of those under #8278, all are *Lepocreadium areolatum* (Linton, 1900) Stunkard, 1969.

The small specimens listed under #8278, from young *M. saxatilis*, 55 to 110 mm in length, are specifically distinct from the single specimen from the same host, designated #8277 in the Helminthological Collection. There are 30 of these small specimens, mounted on a single slide. The description of these worms as given by Linton (1940) is reasonably complete. He gave the size, shape, sizes of suckers and gonads, and disposition of the vitellaria. Two specimens are represented in his Figures 55 and 56. As characteristic features of the species he noted the large size and small number of eggs. He reported that no more than two eggs were present in any worm. Some of the small worms were immature and contained no eggs but eggs were present in very small specimens. The eggs measured 0.10 by 0.06 mm and were partially collapsed.

The specimens have now been restudied. One of the largest of them is shown in Figure 4. It is 0.46 mm in length and is one of two that contains three

eggs; all the other gravid worms have only one or two eggs. The largest juvenile specimen is shown in Figure 3. In it the seminal receptacle is filled with spermatozoa and other specimens of approximately the same size contain a single egg. The small size, large size of eggs, and over-all morphology of these worms are so strikingly similar to the adults of *L. setiferoides* that the identity is apparent. A detailed comparison with the descriptions of Martin and Linton confirm the allocation.

The genus *Lepocreadium* was erected by Stossich (1904) with *Distomum album* Stossich, 1900 as type species. Palombi (1937) reported that *Cercaria setifera* Monticelli, 1914, *nec* Joh. Müller, 1786, is the larva of *L. album*. The life-cycle of a second species, *Lepocreadium pegorchis*, was reported by Bartoli (1967). Many species with divergent morphology have been assigned to *Lepocreadium* and as a result the generic concept has become indefinite and uncertain. Sogandares-Bernal and Hutton (1960) reported on marine species from the North American Atlantic. They stated that the status of these species has been in a state of confusion for the last twenty years. They declared that *L. truttaforme* Linton, 1940 was a composite of at least two different species and redescribed the type specimen from the sculpin, *Acanthocottus octodecimspinosus*, #8276 in the Helminthological Collection of the U. S. National Museum. *Lepocreadium retusum* Linton, 1940 was transferred to *Neolepidapedon*. Two new species, *Lepocreadium caballeroi* and *Lepocreadium opsanusi*, were described. *Lepocreadium caballeroi*, based on a single specimen from *Menticirrhus saxatilis*, is probably identical with *L. areolatum* (Linton, 1900) from the same host and *L. opsanusi* is very similar to and may be identical with *Lepocreadium trulla* (Linton, 1907) Linton, 1910. *Distomum* sp. of Linton (1901, page 458, Fig. 346) from the scup, *Stenotomus chrysops* at Woods Hole was tentatively named *Lepocreadium ovalis* n. sp. by Manter (1931), based on a variety of specimens from the pinfish, *Lagodon rhomboides*, taken at Beaufort, North Carolina. Yamaguti (1958) listed the species as *L. ovale* Manter, 1932 but its validity is suspect. Linton (1940) included *Distomum pyriforme* Linton, 1900 from *Palinurichthys perciformis*, a species he (1901, 1905) reported from other fishes, as a member of *Lepocreadium*. Stunkard (1969a) noted that the specimens listed as *L. pyriforme* by Linton (1940) comprised a heterogenous collection of different species but did not include *Distomum pyriforme* Linton, 1900. He worked out the life-cycle of the last species and showed that it could not be included in the genus *Lepocreadium*. Instead, it was named type of a new genus, *Neopechona*.

Summary

Cercaria setiferoides, an ophthalmotrichocercous distome larva from *Nassa obsoleta*, was described by Miller and Northup (1926). Its life-cycle was reported by Martin (1938) and the excretory system was studied by Peters (1961). Martin found the larvae encysted as metacercariae in the turbellarian, *Procerodes warreni*, and in the annelid, *Spio* sp. The final hosts were small flounders and sand dabs. There are serious disagreements and discrepancies in the three accounts and a redescription of the successive stages in the life-cycle provides new information as well as the correction of previous errors. Experimental infections have disclosed new secondary intermediate hosts and the adult stage is regarded as identical with worms described by Linton (1940) as *Lepocreadium trullaforme* n. sp.

But *L. trullaforme* was suppressed by Yamaguti (1958) as a synonym of *Distomum areolatum* Rudolphi of Linton, 1900, and Stunkard (1969b) redescribed that species as *Lepocreadium areolatum* (Linton, 1900) n. comb. This taxonomic disposition restores the validity of *Lepocreadium setiferoides* (Miller and Northup, 1926) Martin, 1938.

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